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The study of amphipods in rimstone pools of Akiyoshi-do Cave, Japan

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Abstract

Akiyoshi-do Cave is the largest show cave in Japan and has been recognised as a hotspot of cave animals due to their diversity in species. Human-induced alterations in the cave environment have been a significant concern catching the attention of tourists and managers. Previous studies indicated water quality alteration induced by tourism could affect the population densities of amphipods. However, no study went further than qualitative observation in terms of human impacts. This study targets two amphipods living in Akiyoshi-do Cave, *Pseudocrangonyx akatsukai* and *Gammarus nipponensis* and measures water characteristics in which they live. Results show that the population densities of the amphipods have decreased compared to the 1970s. Their living habitat has changed, probably induced by tourism.

Keywords

show cave, human impact, amphipod, water quality, protection

Introduction

In Japan, caves have a long history of being recognised as places where gods live, but when civilisation was developed, tourism use of caves began and now there are over 100 show caves in Japan (Zaizenji et al. 1993; Itoda and Goto 2018).

Akiyoshi-do Cave is famous as one of the largest show caves in the country, and in the last years the annual number of tourists reached about 500,000. The area has

been actively explored and researched since the early 19th century and the fields of research include geography, hydrology, biology, archaeology and humanities (Yamaguchi Caving Club 1992). Figure 1 shows the number of studies concerning Akiyoshi-do Cave made between the 1900s and the 2010s. Fifty-four of more than 120 studies targeted cave animals. They include: reports of new species, behavioural characteristics of bats, microbial community analysis, and others (Uemura 1941; Kuroda and Watanabe 1958; Miyoshi 1958; Okafuji 1958; Ueno S-I 1958a, b; Uchida and Kuramoto 1968; Hori et al. 2008). The Chimachida rimstone pools are one of the main tourist spots in the cave, rich in cave animals, and their water quality and biological diversity have been investigated by Uéno (1933) and Wakisaka et al. (1962).

A crustacean amphipod, *Pseudocrangonyx shikokunis* is a troglobite described in the first biological paper focusing on Akiyoshi-do Cave, which was collected from the Chimachida rimstone pools (Uéno 927). The amphipod was revealed to be a new species and was renamed *Pseudocrangonyx akatsukai* Tomikawa & Nakano, 2018. *P. akatsukai* contributed to the further understanding of the biogeographical history of *Pseudocrangonyx* in western Japan. The water quality of the Chimachida rimstone pools was measured in 1931 and 1932 and indicated a water temperature of 12.7–16.0 °C, pH of 7.7–8.0, and an alkalinity of 3.8–4.3 meq/ml (Uéno 1933). Calcium and magnesium concentrations of the pools were measured as 63.7 mg/l and 2.9 mg/l, respectively, in 1962 (Wakisaka et al. 1962). Another resident of the Chimachida rimstone pools is *Gammarus nipponensis* Uéno, 1940, which is a troglophile originating outside the cave. The population density fluctuations of *P. akatsukai* and *G. nipponensis*, in the Chimachida rimstone pools, were surveyed for 3 years and 9 months from April 1971 to January 1975. The results showed that their population densities exhibited seasonal changes: 1–13 ind/m^2 for *P. akatsukai* and 1–990 ind/m^2 for *G. nipponensis*. It was suggested that the amphipods were affected by the number of tourists, increasing in summer and decreasing in winter (Nakamura and Kuramoto 1978). However, it was

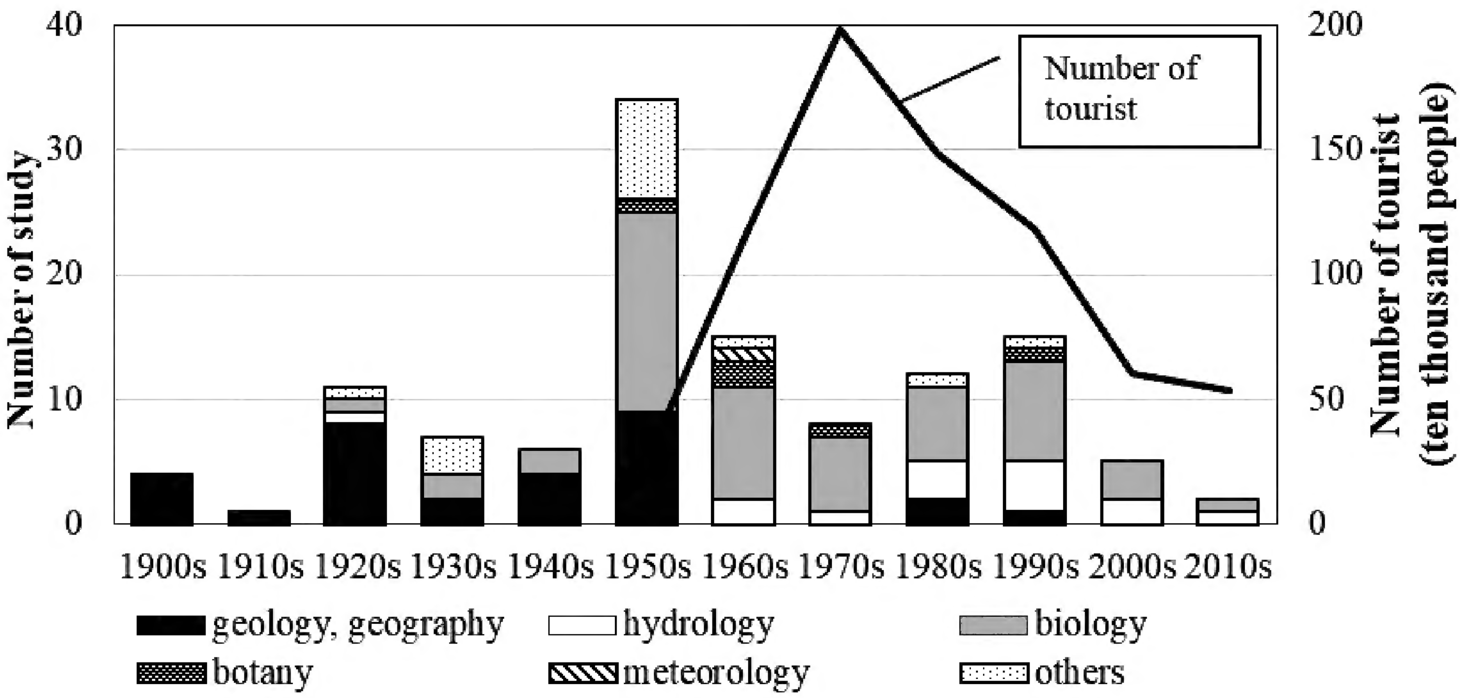

Figure 1. The number of existing studies and their breakdown of academic fields for each decade.

not mentioned in which pool, among the approximately 500 Chimachida rimstone pools, the water quality was measured by Uéno (1933) and Wakisaka et al. (1963). This means that the habitat of *P. akatsukai* and *G. nipponensis* lacks reliable water quality data. In addition, the most recent data on water quality is absent because water monitoring has not yet been installed. Even though the fluctuations of the population density of the two amphipod species depending on organic matter input was suggested by Nakamura and Kuramoto (1978), it remains unknown if and how the amphipods respond to water characteristics.

The objective of this study is to reveal the quality of the amphipods' habitat and to observe their population and distribution while taking tourism impacts into consideration.

Materials and methods

Study site

Akiyoshi-do Cave is a show cave located at 34°13.44'N, 131°18.14'E and roughly at 84 m above sea level (Figure 2). It is located in Akiyoshi-dai Plateau National Park, Yamaguchi Prefecture in Japan, and is known as one of the most visited show caves in the country due to its development, big stalactites and biodiversity of cave inhabitants. The cave is on the lists of Natural Monuments (since 1922), Special Natural Monuments (since 1955), Ramsar Convention Wetlands (since 2005) and Japan Geoparks (since 2015) and offers people learning experiences in a natural environment.

The Chimachida rimstone pools are one of the main tourist spots in Akiyoshi-do Cave located about 330 metres from the main entrance. They are composed of approximately 500 large and small pools arranged within a diameter of 20 m (Figure 3). The upper part is in contact with the tourist trail, has a gentle slope over the lower part and water eventually joins the cave river towards the main entrance, which is southwards. There is a wall with a film of flowing water on the opposite side across the tourist trail and the water is transported by a pipe under the tourist trail and discharged into the pools. In addition, pumped-up cave river water is continuously provided to the pools; this water supply started on 19 October 1971 with the aim of improving the water quality, in response to the fact that the pools' contamination had become noticeable. Spring water at the bottom of some pools is another source of water supply to the pools. Dripping water from the ceiling is another supply source but much smaller compared with the others.

Ten pools were selected from the Chimachida rimstone pools and named CH1 to CH10. Visual observations were made of the colour of the pool bottom, the particle size of sediments, the presence of human-related debris and other features, for qualitative evaluation of each pool on 16 November 2015. The water quality of pools and the population densities of the two amphipod species were measured/observed on 17 November 2015, 17 February, 17 May and 8 August 2016.

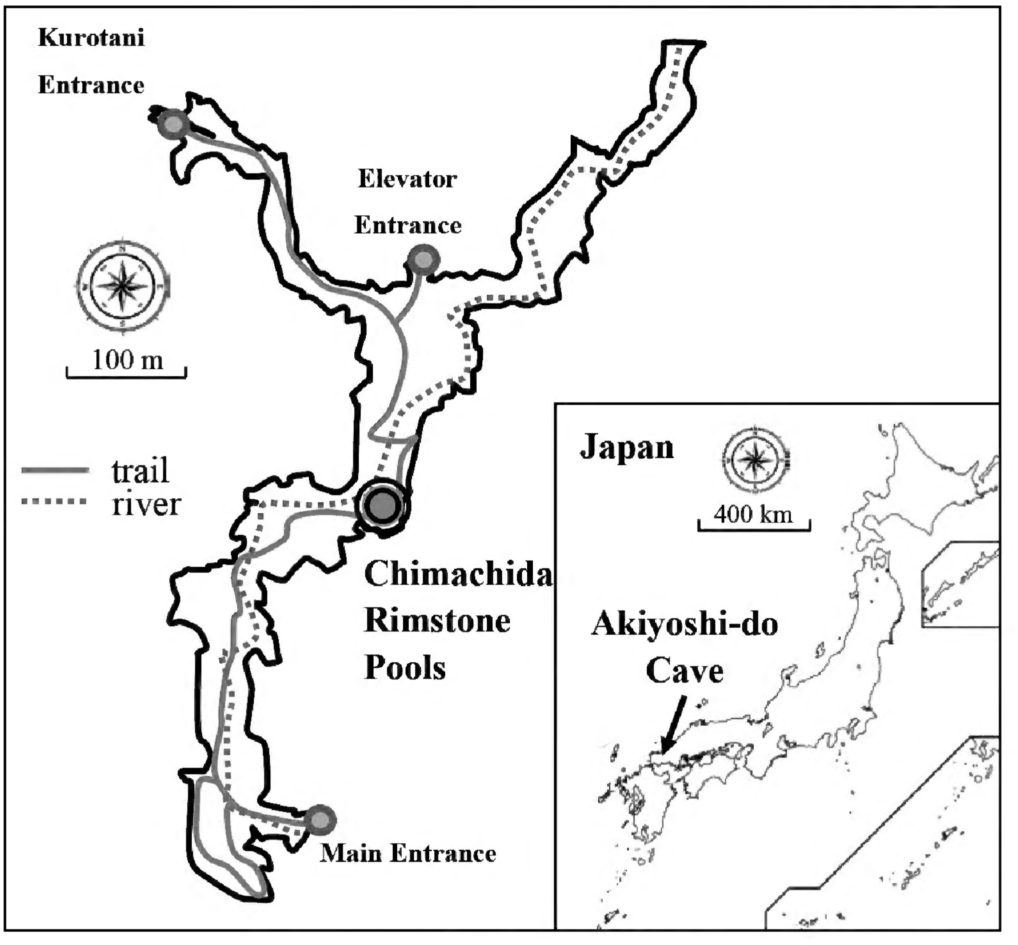

Figure 2. Location of the Akiyoshi-do Cave in Japan and of the surveyed pools inside the cave. The cave river flows to the south. The map of Akiyoshi-do Cave was drawn based on karusuto.com (https://akiyoshido.karusuto.com/html/guide/).

Nakamura and Kuramoto (1978) classified the pools into three types, based on the sediment of the pool bottom and the presence/absence of the amphipods. Type A pools were defined by the deposition of reddish-brown clay which suggested little inflow of organic matter. Type B pools were characterised by the deposition of black-brown clay, suggesting a large influx of organic matter. Type C pools were characterised by a larger amount of polluted black-brown clay deposition compared with type B pools. In this study, pools were classified in the same way, plus, the water source was considered. The small differences between the previous and our classification concerned the pools unselected in the previous study, the improved condition of the pool bottom, thus reclassifying some from type C to B, and worsened condition of the pool bottom, thus reclassifying some from A to C.

The 10 study pools can be classified into three major types, according to the type of water supplied to the pools, either water on the walls across the tourist trail or pumped-up cave river water. The first type, named type A pool, are pools that receive a larger amount of water from the wall, this being transported under the tourist trail and supplied to the pool, and this corresponds to CH1-5. Another type, named type B pool, are pools which receive a large amount of pumped cave river water, and this corresponds to CH6-9. Finally, there is a type C pool characterized by the absence of water exchange, and it corresponds to CH10. In addition, CH10 has a higher elevation of the pool bottom than adjacent pools and is isolated by a rimstone wall thicker than 5 cm.

Figure 3. The main entrance of Akiyoshi-do Cave (left) and the Chimachida Rimstone Pools (right).

The aquatic fauna in pools

P. akatsukai is a troglobite, with white body, reduced eyes, developed sense of touch, and is adapted to an oligotrophic environment (Uéno 1927; Tomikawa and Nakano 2018) (Figure 4). Many of these amphipods live in places where the flow of water is slow, such as pools and underground rivers. *G. nipponensis* is a troglophile that lives both inside and outside of caves, prefers organic matter more than *P. akatsukai* and has no preference for water flow speed (Figure 4).

Field observations and measurements

A 50 cm square quadrat was created in order to measure the population densities of *P. akatsukai* and *G. nipponensis*. The quadrat was placed in the pools and the number of amphipods was counted with the naked eye.

Water temperature, pH and electric conductivity were measured on site using a HORIBA handy meter (model SSS054, D-54). The quantity of dissolved oxygen was measured using a HORIBA handy meter (model SS054, D-55s).

Experiments in laboratory

Water samples were collected on site using two 250 ml water bottles per site, placing them in a cool box at below 10 °C and transported to the laboratory.

The alkalinity (C) was determined by titration method. Chemical oxygen demand (COD) (mg/l) was determined by the potassium permanganate titration method. Total nitrogen (TN) was measured by Merck's pack test. The total phosphorus content (TP) was also determined by Merck's pack test.

The number of all bacteria in the water sample, which is known as one of the indicators of water pollution, was determined. A test tube containing 9 ml of sample water was prepared and 1/10 of a formalin solution 1 ml was added and mixed well to fix bacteria in the sample. Bacteria are stained by adding DAPI (4', 6-diamidino-2-phe-

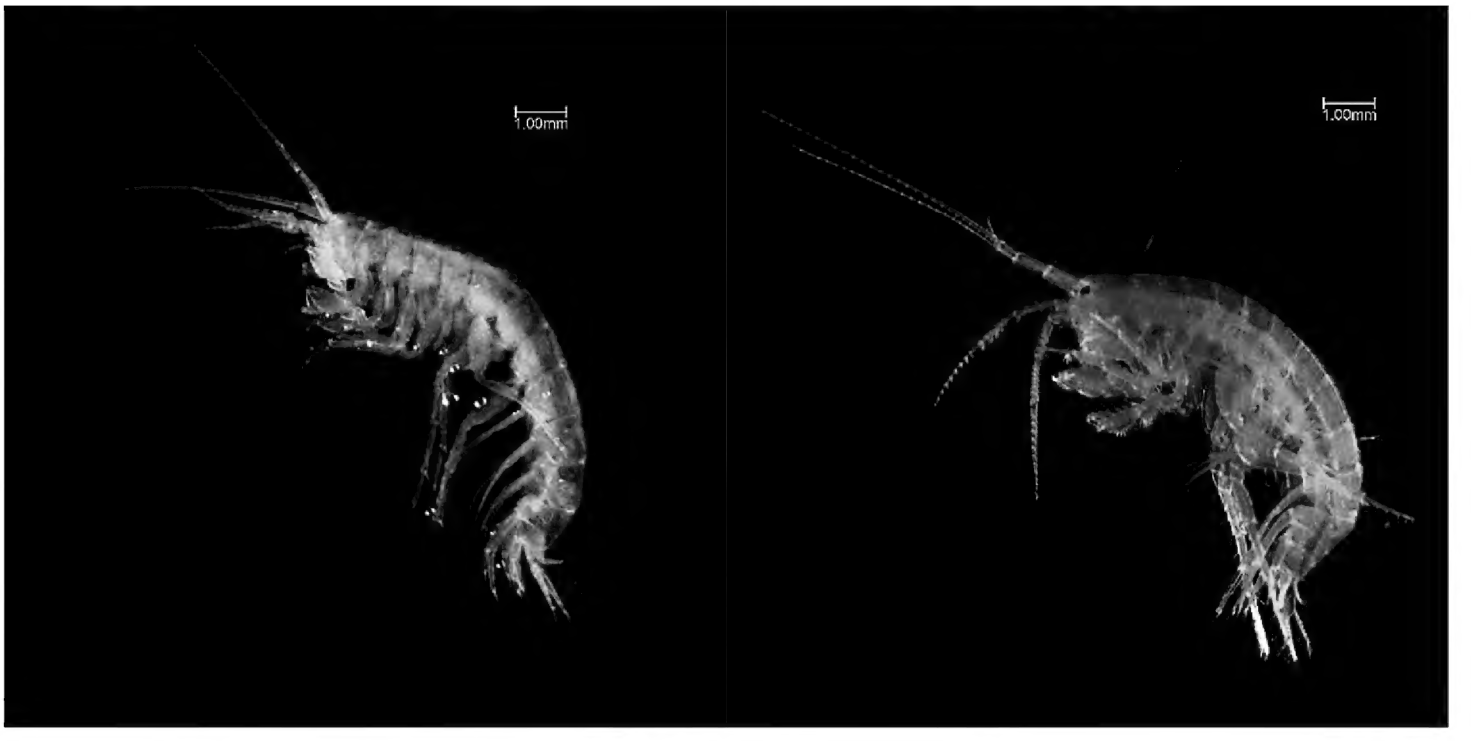

Figure 4. *Pseudocrangonyx akatsukai* (left) and *Gammarus nipponensis* (right).

nylindole) nucleic acid stain, water is filtered through an anopore inorganic membrane filter produced by Whatman plc and the total number of bacteria was counted using a fluorescence microscope.

The number of viable bacteria is also a biological index of water pollution, as well as the total number of bacteria. Undiluted sample water, 1/10 diluted sample water and 1/100 diluted sample water were placed in 1/10 Nutrient Broth, 1.5% agar medium and cultured for 3 days in an incubator set at 20 °C. Two plates were made for each dilution level. The experiment was performed on a clean bench environment. Bacterial colonies were marked on plates by dots with a pen until the cultivation period ended.

Statistical analysis

Canonical Correlation Analysis was performed with software R in order to clarify the correlation between each of the water quality parameter and the two amphipod species. Total nitrogen and total phosphorus as nutrients for the amphipods, and both total number of bacteria and total number of viable bacteria as indicators of water pollution, were used in the analysis.

Results

Physicochemical characteristics of pools

The results of macroscopic inspection of the pools are shown in Figure 5 and Table 1.

Table 1 shows contaminants and other characteristics of the three types of pools. In the type A pools, no contaminants such as green algae or hair, were observed or there

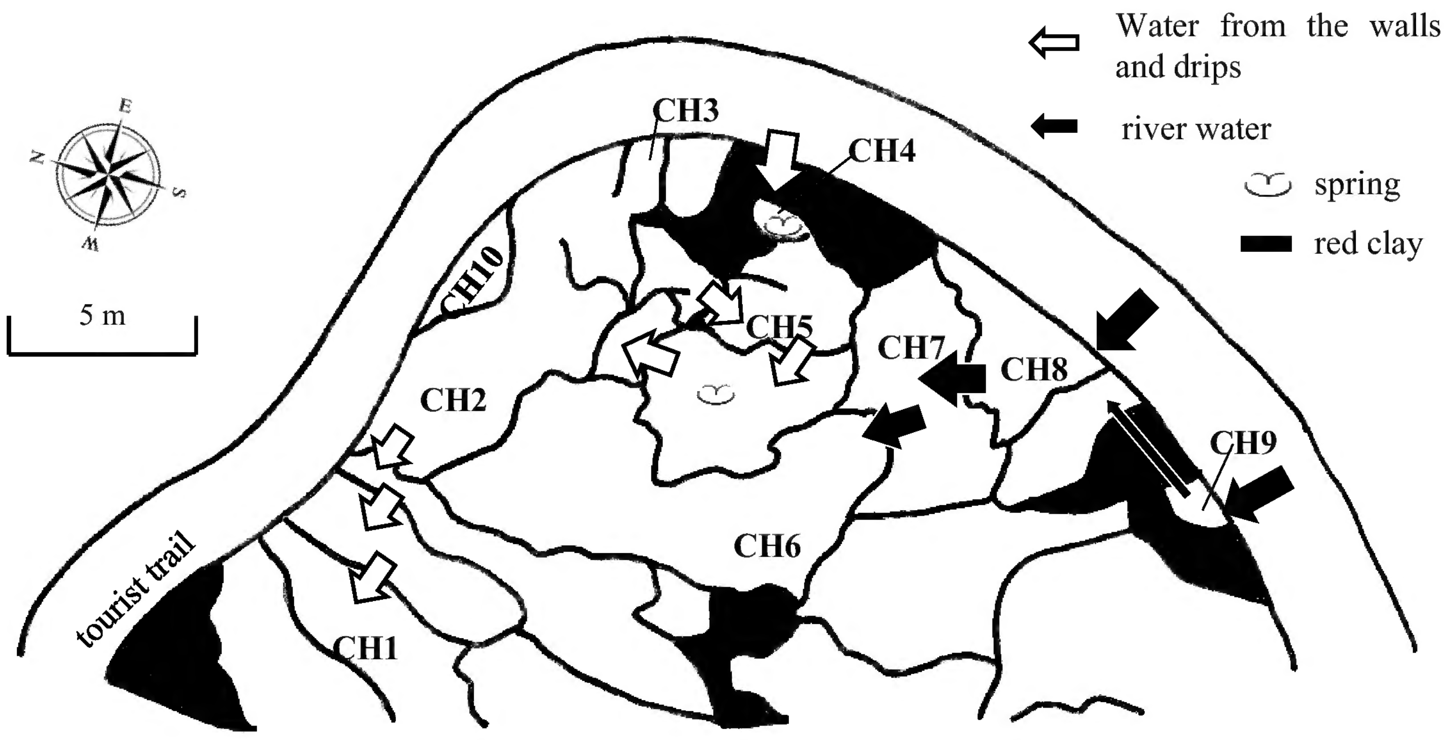

Figure 5. Pools with arrows pointing the flowing directions. Water from the walls and drips was supplied to CH4 and water flowed to CH2 and CH1 via CH5. Pumped cave river water was supplied to CH8 and CH9 and water flowed to CH6 and CH7.

Table 1. Results of macroscopic observation of the analysed pool in Akiyoshi-do Cave.

Name	Type	Water resource	Bottom color	Grain size	Contamination	Other features
CH1	A	transported upwelling water	ochre	sand, clay	–	–
CH2	A	transported upwelling water	ochre	gravel, sand, clay	algae, hair	–
CH3	A	transported upwelling water	blackish	sand, clay	algae, hair, plastic	–
CH4	A	transported upwelling water	reddish	clay, sand	–	considerable sand surrounding the spring
CH5	A	river water, srping water, transported upwelling water	entirely reddish, especially the surrounding of the spring	clay	–	
CH6	B	river water, spring water	reddish ochre	clay	–	cave coral develops
CH7	B	river water	black	sand, clay, gravel	algae, hair, woodchip	a fresh water crab was observed
CH8	B	river water	deep black	clay	considerable algae, plastic	–
CH9	B	river water	deel black	clay, sand	big plastic chips, algae, cans scrap, lint	–
CH10	C	transported upwelling water	blackish ochre	grave, sand, clay	algae, hair, woodchip	isolated from surrounding pools

were in very small amount compared with the type B pools. By contrast, in the type B pools, contaminants, such as hair and plastic fragments, were particularly noticeable and abundant green algae had developed. On the other hand, in the type C pool, green algae development and contaminants, such as hair and wood chips, were more prominent than in type A and B pools.

Laboratory measurements

Table 2 shows the mean values of the water parameters across the Chimachida rimstone pools. The water temperature was slightly lower than 17 °C, the mean temperature of

the atmosphere in Akiyoshi-do Cave. The values of total nitrogen and total phosphorus of type A, B, C pools in each survey are also represented in Table 2. The mean values of total phosphorus were in increasing order in type A, type B and type C pool, of 0.05 mg/l, 0.11 mg/l and 0.17 mg/l, respectively. The mean values of chemical oxygen demand were 0.2 mg/l for both type A and type B pools, and 0.6 mg/l for the type C pool (Table 2). The type C pool showed the total number of bacteria of 1.3×10^6 Cells/ml and the total number of viable bacteria of 1.3×10^4 CFU/ml, both exceeding the mean values of the type A and B pools. The mean values of the total number of bacteria were 5.7×10^5 Cells/ml and 9.8×10^5 Cells/ml for type A and B pools, respectively. The mean values of the total number of viable bacteria were 3.7×10^3 Cells/ml and 1.1×10^4 Cells/ml for type A and B pools, respectively.

Population densities and distribution of amphipods

Table 3 shows the population densities of *P. akatsukai* and *G. nipponensis* of all surveys in this study. The population densities of both species varied from 0 to 10 ind/m^2. The population densities of *P. akatsukai* were 0–8 ind/m^2, 0–6 ind/m^2, and 0–1 ind/m^2 for type A, B, and C pools, respectively. The population densities of *G. nipponensis* were 0–10 ind/m^2, 0–4 ind/m^2, and 0–20 ind/m^2 in type A, B, and C pools, respectively. Table 4 and Figure 6–8 show the comparison between population densities of 1970s and present. The maximum population densities of *P. akatsukai* in type A pools in the 1970s was 12 ind/m^2, and that of the present was 12 ind/m^2. The maximum population densities of *G. nipponensis* in type A pools in the 1970s and the present were both 8 ind/m^2. The maximum population densities of *G. nipponensis* decreased from the 1970s to the present in type B and C pools: from 260 to 20 ind/m^2, from 935 to 10 ind/m^2, respectively.

Seasonal changes in population densities of amphipods

Number of both *P. akatsukai* and *G. nipponensis* increased in autumn, but decreased in winter (Table 3). In type B pools *P. akatsukai* increased in spring and summer, and *G. nipponensis* increased in autumn and winter (Table 3). In the type C pool, *P. akatsukai* was not observed, whereas *G. nipponensis* was observed in summer and autumn (Table 3).

Canonical Correlation Analysis

The analysis results are shown in Figure 9. *P. akatsukai* had a relatively strong negative correlation with total nitrogen. *G. nipponensis* had a positive correlation with the total phosphorus, the total number of bacteria and the total number of viable bacteria.

Table 2. Mean water characteristics of the 4 surveys (17th November 2015, 17th February 2016, 17th May 2016, and 8th August 2016) in the different pools of Akiyoshi-do Cave. *PA*: *Pseudocrangonyx akatsukai, GN*: *Gammarus nipponensis*, WT: water temperature, EC: electric conductivity, DO: dissolved oxygen, ALK: alkalinity, TN: total nitrogen, TP: total phosphorus, COD: chemical oxygen demand, TB: total number of bacteria, TVB: total number of viable bacteria.

Pool type	A						B					C	Mean total
	CH1	CH2	CH3	CH4	CH5	mean	CH6	CH7	CH8	CH9	mean	CH10	
WT (°C)	14.9	14.8	14.9	14.9	14.9	14.9	14.8	14.9	15	14.8	14.9	14.9	14.9
pH	7.6	7.6	7.6	7.7	7.8	7.7	7.8	7.8	7.9	7.9	7.9	7.8	7.8
EC (mS/m)	27.8	28.3	31	26.5	28.5	28.4	28.2	26.5	26.9	27.6	27.6	26.6	27.8
DO (mg/L)	8.7	7.7	7.3	8.3	8.1	8.0	8.8	7.3	8.2	8	8.1	7.4	8.0
ALK (meq/L)	3.0	2.9	3.1	2.6	2.8	2.9	2.8	2.7	2.6	2.7	2.7	2.8	2.8
TN (mg/L)	1.1	1.4	1.4	1.4	1.3	1.3	1.4	1.2	1.1	1.4	1.3	1.0	1.3
TP (mg/L)	0.10	0.02	0.08	0.04	0.03	0.05	0.13	0.19	0.05	0.08	0.11	0.17	0.1
COD (mg/L)	0.2	0.1	0.1	0.4	0.3	0.2	0.3	0.3	0.1	0.2	0.2	0.6	0.3
TB (Cells/mL)	2.3×10^5	6.3×10^5	6.4×10^5	4.3×10^5	9.1×10^5	5.7×10^5	5.8×10^5	9.4×10^5	6.9×10^5	1.7×10^6	9.8×10^5	1.3×10^6	8.1×10^5
TVB (Cells/mL)	3.4×10^3	3.3×10^3	1.5×10^3	2.7×10^3	7.4×10^3	3.7×10^3	1.0×10^4	9.1×10^3	8.2×10^3	1.7×10^4	1.1×10^4	1.3×10^4	7.6×10^3

Table 3. Population densities of *Pseudocrangonyx akatsukai* and *Gammarus nipponensis* for both different pool types and periods in Akiyoshi-do Cave.

		Nov-15		Feb-16		May-16		Aug-16	
		P. akatsukai	*G. nipponensis*	*P. akatsukai*	*G. nipponensis*	*P. akatsukai*	*G. nipponensis*	*P. akatsukai*	*G. nipponensis*
Type A pools	CH1	6	0	1	0	0	1	2	0
	CH2	8	8	2	2	2	1	4	0
	CH3	6	2	2	0	0	0	2	0
	CH4	0	0	4	0	10	0	6	0
	CH5	6	4	0	0	2	0	2	0
Type B pools	CH6	6	2	1	0	1	0	0	0
	CH7	0	4	0	1	1	0	2	0
	CH8	0	0	0	1	1	1	0	4
	CH9	0	20	1	1	0	4	0	8
Type C pools	CH10	0	10	0	0	0	0	0	6

Table 4. Mean population density of amphipods measured in 1970's and during this study in Akiyoshi-do Cave. Numbers in the parentheses indicate the numbers of data taken.

Pool type	Amphipods	1971–1975 (45)			2015–2016 (4)		
		min	max	mean	min	max	mean
A	*Pseudocrangonyx akatsukai* (ind/m^2)	1	12	5.1	0	10	3.3
	Gammarus nipponensis (ind/m^2)	3	8	41.9	0	8	0.9
B	*Pseudocrangonyx akatsukai* (ind/m^2)	N.D.	N.D.	N.D.	0	6	0.8
	Gammarus nipponensis (ind/m^2)	18	269	112.6	0	20	2.9
C	*Pseudocrangonyx akatsukai* (ind/m^2)	N.D.	N.D.	N.D.	0	0	0.0
	Gammarus nipponensis (ind/m^2)	8	935	304.9	0	10	4.0

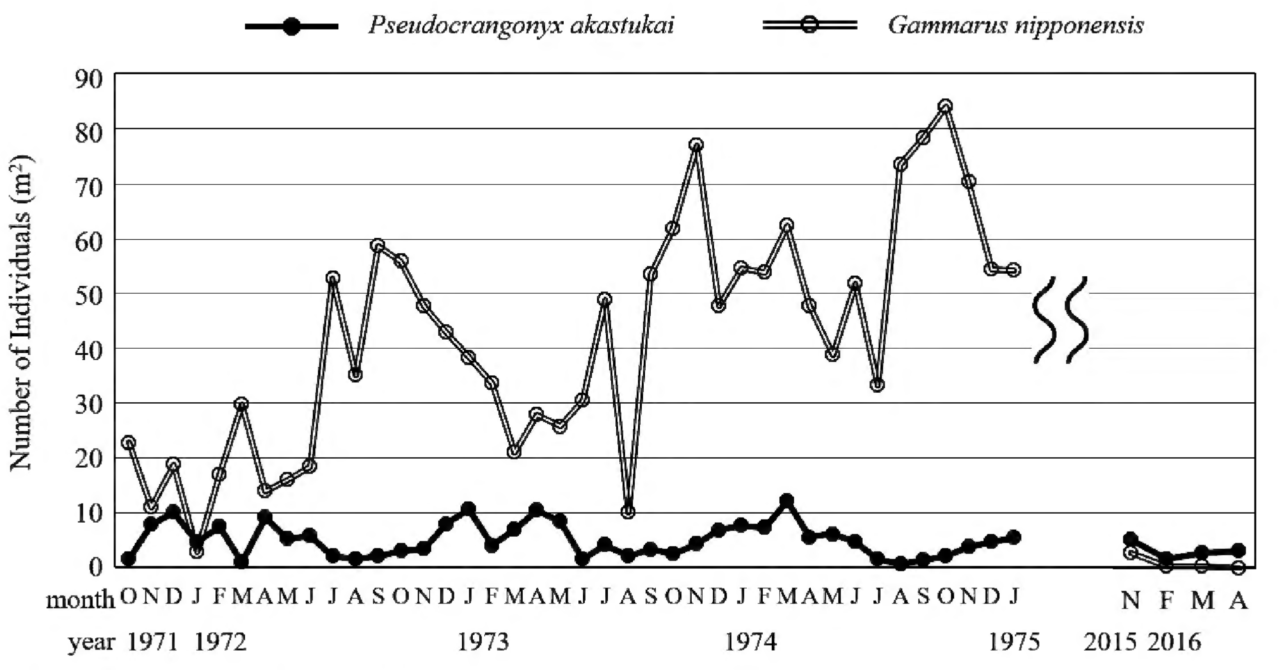

Figure 6. Fluctuation of the population densities of *Pseudocrangonyx akatsukai* and *Gammarus nipponensis* in the type A pools (1971–1975, 2015–2016).

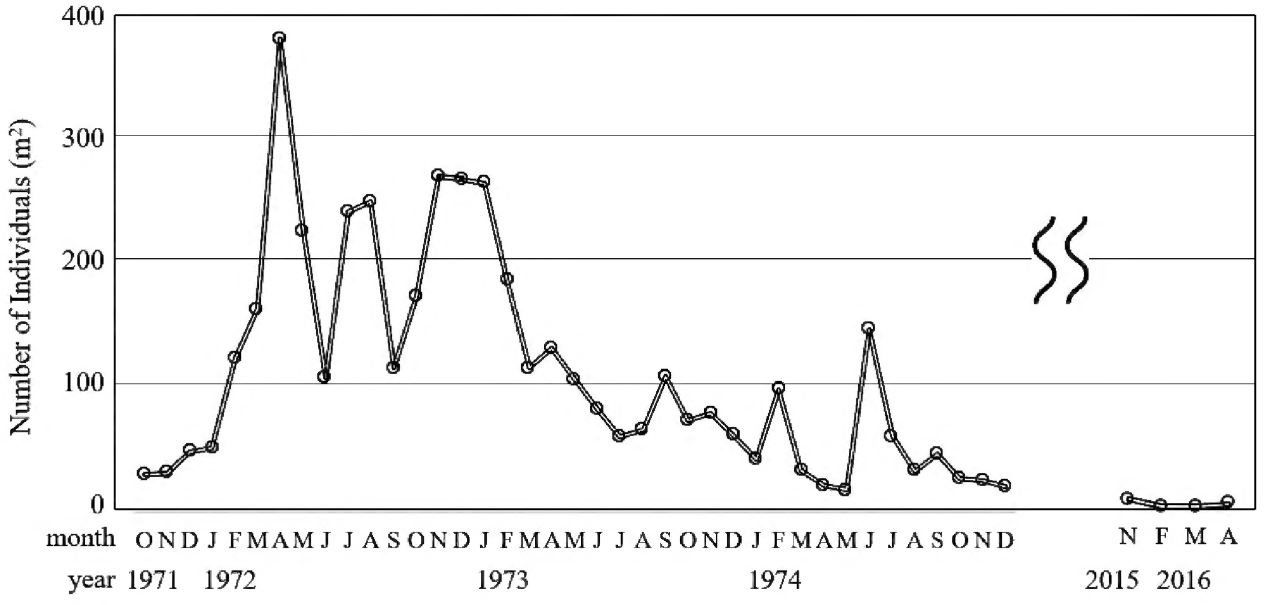

Figure 7. Fluctuation of the population density of *Gammarus nipponensis* in the type B pools (1971–1975, 2015–2016).

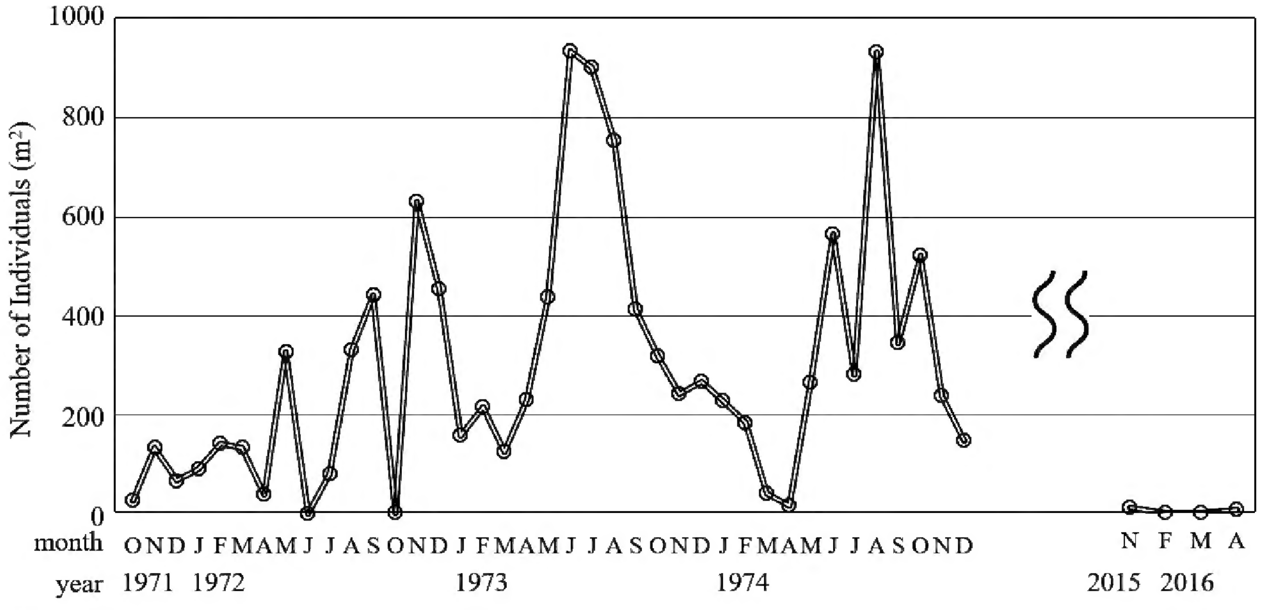

Figure 8. Fluctuation of the population density of *Gammarus nipponensis* in the type C pools (1971–1975, 2015–2016).

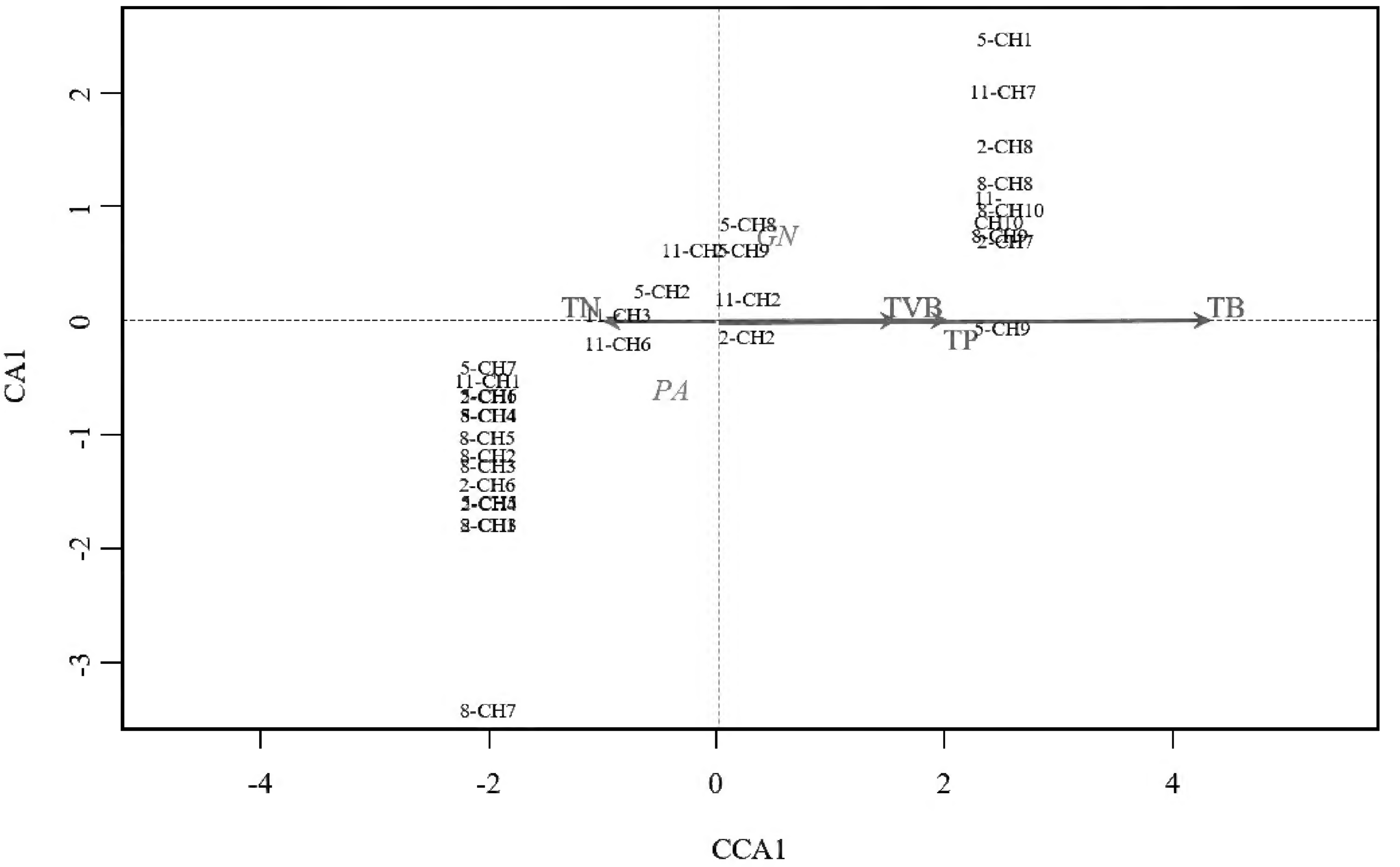

Figure 9. Result of CCA analysis on the amphipod abundances and water characteristics. TN: total nitrogen, TP: total phosphorus, TB: total number of bacteria, TVB: total number of viable bacteria. Other symbols mean pools surveyed in each season by "month-pool number": for example, 5-CH1 corresponds to CH1 pool surveyed in May.

Discussion

Correlation between amphipods and water quality

The obtained correlations corroborate with the findings reported by Nakamura and Kuramoto (1978) that *P. akatsukai*, a troglobite, does not like the nutrient-rich environment (total nitrogen) and, hence, it was suggested that the increase in organic matter contamination caused by tourism could reduce their habitat availability. On the other hand, the population density of *G. nipponensis*, a troglophile, increased as the number of microorganisms and the amount of nutrient (total phosphorous) increased. However, it was observed that the population density of *G. nipponensis* decreases as water pollution progresses (Nakamura and Kuramoto 1978). It, thus, might indicate a threshold for the concentration of organic matter in the water in which *G. nipponensis* can live.

Seasonal changes in population densities of amphipods

The study of Nakamura and Kuramoto (1978) showed that the population density of *P. akatsukai* increased in winter and decreased in summer and that of *G. nipponensis* increased in summer and decreased in winter, in type A pools. The present study showed different results in type A and B pools.

Habitat ranges of amphipods – Comparison with the 1970s results

It was stated that *P. akatsukai* did not inhabit pools CH7-9 in the 1970s. In the four surveys conducted in this study, *P. akatsukai* was confirmed in those three pools. Pumped river water supply started in 1971, possibly contributing to the habitat expansion of *P. akatsukai*. On the other hand, in CH10, the type C pool, formerly classified as a type A pool, *P. akatsukai* was not found in the present survey. The CH10 pool is surrounded by a thick wall of 5 cm or more and water exchange was not confirmed. No other water supply exists for CH10. This pool is located at the top of Chimachida rimstone pools and is one of the pools with the highest frequency of contact with tourists. Therefore, relatively large amounts of tourist-derived organic matter are supplied and eutrophication progresses. This can be the reason why *P. akatsukai*, which is known not to inhabit eutrophic environments, retreated from this pool.

Population density of amphipods – Comparison with the 1970s results

The population density of both *P. akatsukai* and *G. nipponensis* in any type of pool decreased compared to the 1970s. In the absence of population density data prior to the 1970s makes it difficult to discuss the impact of tourism development in Akiyoshi-do Cave.

Akiyoshi-do Cave experienced the highest number of tourists in the 1970s and has shown a gradual decreasing trend since then to the present, but the population density of *P. akatsukai* and *G. nipponensis* became significantly smaller.

Impacts on the Chimachida rimstone pools

It is difficult to conclude that the source of organic matter contamination to the Chimachida rimstone pools is limited to cave tourism. The tourist activities on the surface where Akiyoshi-do Cave is developing, the so-called Akiyoshi-dai Plateau, also take part in environmental alteration of the water inside the cave. There is a museum, observation decks and shops located on the plateau within the range of the catchment area of Akiyoshi-do Cave. The sewage from these tourist facilities is transported to the basement through the underground sewer and treated. If sewage leaks out it can contaminate the Chimachida rimstone pools. In the 1990s, the sewer pipe was damaged, contaminating the upwelling water and creating obnoxious odours at another place not connected to the Chimachida rimstone pools in Akiyoshi-do Cave. Kuramoto and Nakamura (1995) and Kuramoto (1995) conducted a survey to find the influence the leaked sewage had on the fauna in Akiyoshi-do Cave. As a result, they found that many of the subterranean animals that were identified at the time of the survey by the Japan Cave Groundwater Research Association (1957) were observed, even in 1995. In addition, organic matter originating from animal manure can reach Akiyoshi-do Cave from poultry farms and an abandoned ranch in the catchment area of Akiyoshi-do

Cave. Kawano and Fujii (1985), Yoshimura and Inokura (1992) and Haikawa (2006) reported the water system of Akiyoshi-dai Plateau National Park. Their tracer experiments gave no details on the inflows that reach the pools in the Akiyoshi-do Cave.

Conclusion

This study focused on amphipods living in the Chimachida rimstone pools for the first time after 40 years and discovered that their habitat range and population density are different from the past. The habitat range of *P. akatsukai* expanded, while that of *G. nipponensis* narrowed. The population density of both *P. akatsukai* and *G. nipponensis* decreased. The seasonal variation in the population density of the amphipods was not confirmed in this study. As the observation period was of about 1 year which was shorter than the survey done in the 1970s, it needs more data to describe seasonal changes better. The habitat range and the population density of the amphipods should be monitored regularly. There is also need for hydrological investigations at the places suspected to be the origins of organic matter for the Chimachida rimstone pools, in the future.

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